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Effect of Pineapple Storage duration on the Quality of Bromelain age Duration of Pineapple on the Quality of the Bromelain Produced

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Pineapples are an abundant source of bromelain, however, during the distribution, the storage process can affect the bromelain content of pineapples, including rotting fruits. Those that have rotted are usually just thrown away without any further treatment. Therefore, this research was conducted to determine the effect of the distribution or storage time of pineapple on its bromelain content. Harvested pineapples are stored at room temperature for 9 days. Every three days, the pineapples were then sampled to see their bromelain content and profile. Crude enzyme yield, bromelain levels, crude enzyme activity are the main parameters in this research. From the research results, it can be observed that the longer the pineapple was stored, the greater the crude bromelain yield obtained. However, even though the yield was increasing, it was known that the levels of proteins and bromelain as well as the activity of the crude bromelain were actually getting smaller. The protein content of the crude bromelain decreased from 18.76 g/mL to 6.02 g/mL. Furthermore, the bromelain content also decreased from a content of 2.2435 mg/mL to 0.5175 mg/mL. Crude bromelain activity is directly proportional to bromelain levels, namely decreasing from 73.73 U/mg decreasing to 27.60 U/mg. It can be concluded from this research that the yield value is not directly proportional to the properties and levels of crude enzymes produced from pineapples that are stored for several days. Even though the pineapple had been stored for 9 days, the bromelain yield and activity obtained were still high. The results of this research showed that unsold pineapple and have started to rot can have its enzymes extracted as one of the steps that can be taken in processing pineapple products, and producers or farmers do not incur significant losses.

Keywords: Bromeliaceae, waste management, enzyme activity, industrial practically, postharvest, proteolytic.

INTRODUCTION

Pineapple (*Ananas comosus*) is plant belonging to Bromeliaceae family which is a commercially grown species, and it is abundant in nutrients (Abraham *et al.,* 2023). In addition, pineapple also plays an important role in international trade after bananas and oranges (María Gloria Lobo, 2017). In Asia, one of the highest pineapple producing countries is Indonesia, which reaches 1,805,506 tons/years (bps.go.id, 2019).

Pineapples also produce quite a lot of waste in the form of stems, leaves, skins, stumps, and rotten fruit. Observations in the field reveal that pineapple fruits begin to rot on the 7th to 9th day of storage, this prompted it to be discarded. In the market, this is allowed to happen and is no longer considered important by sellers or farmers. However, recently, the added value of pineapple waste has been scaled up to a laboratory scale. Products that can be processed include cellulose and hemicellulose (Suryanto *et al.,* 2023; Syukri *et al.,* 2024). The chemical industry also utilizes pineapple residues or waste to produce ethanol, methane, citric acid, and several types of antioxidant compounds (Imandi *et al.,* 2008).

In addition, pineapple fruit also contains bioactive compounds, especially proteolytic enzymes, which are an alternative to waste treatment (Campos *et al.,* 2019). The

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proteolytic enzyme found in pineapple is bromelain. It can be found in various parts of the pineapple (Schieber, 2019; Ketnawa & Rawdkuen, 2011). Commercially, bromelain is obtained from pineapple stems and fruits (Misran *et al.,* 2019).

Bromelain is a group of proteases that have the function of digesting proteins and has promising applications in several food industries, such as tenderizing meat, preventing browning in the bread-making industry, helping to accelerate the fermentation process of fish sauce, protein hydrolysates, and alcohol manufacturing processes (Arshad *et al.,* 2014; Nanda, *et al.,* 2020). In addition, it has been applied in the beverage industry (Ketnawa and Rawdkuen, 2011), the pharmaceutical and medical industries (Dhandayuthapani *et al.,* 2012), and cosmetics (Hikisz and Bernasinska-Slomczewska, 2021). More than 60% of its total sales are biochemical products, indicating proteases are important in major industries (Corzo *et al.,* 2012). This suggests that bromelain is important in major industries.

Bromelain activity is an essential metric. Several parameters can affect bromelain activity, including enzyme supply, pH, temperature, substrate, and inhibitor (Chobotova *et al.,* 2010). According to Liang *et al.* (2012), Fe^{3+} and Cu^{2+} ions suppress bromelain activity. However, the effect of pineapple fruit storage time on bromelain characteristics is still unknown.

The importance of bromelain, the present of this enzyme would undoubtedly decrease during the harvesting process of pineapple before reaching the hands of customers (Antoniolli *et al.,* 2007; Rini *et al.,* 2021; Azima *et al.,* 2016; Syukri *et al.,* 2013). This could be related to a physiological mechanism, specifically the evaporation of water in pineapples, which causes them to shrink (Martinez-Ferrer *et al.,* 2002; Azima *et al.,* 2018). In order to ensure that decaying pineapple fruit can still be valued for bromelain production, it is critical to evaluate the bromelain level of pineapple fruit during the storage process on 0, 3, 6, and 9 days. This is based on previous field observations that have been conducted in local farmer in Indonesia.

MATERIALS AND METHODS

This research was carried out from January 2023 to June 2024. This research was conducted at The Educational Laboratory of Department of Food Technology and Agricultural Products at Andalas University and the Food Distribution Engineering Laboratory at Gifu University, Japan.

***Materials*:** The Queen variety of pineapple was used in this study.

***Crude Bromelain Extraction Process (Modification of Soares et al., 2011)*:** In this study, the pineapple variety used was queen. The pineapple used was three months old with a sufficient level of maturity. After harvesting, they were cleaned and all parts such as stems and leaves were separated. Then the pineapple is stored periodically for 0, 3, 6 and 9 days.

A total of 1 kg of pineapple was prepared for the bromelain extraction process. The fruit was peeled and the pulp was ground. the juice was squeezed using a filter cloth. The filtered juice was then mixed with 90% ethanol in a 1:1 ratio to isolate the crude bromelain. The mixture was left for 24 hours at 4°C. The precipitate formed is bromelain paste. The precipitate was then separated and the remaining ethanol was evaporated at room temperature. The bromelain paste is then stored at 4°C until use.

Yield: The yield of raw bromelain is measured by weighing the amount of paste produced and comparing it with the amount of initial raw material.

***Determination of Proteolytic Activity*:** This analysis was carried out using the spectrophotometric method. Tyrosine standards have been used by making 1000 ppm solutions. Then standard series solutions were made with series 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 ppm. The absorbance of the standard solution was then analyzed with a spectrophotometer with a wavelength of 275 nm. A standard curve was created to see the linearity function of the concentration and absorbance of tyrosine standards. The sample was mixed with 1 mL of 1% casein, 1 mL of 0.1% crude bromelain and 2 mL of pH 7 buffer solution in one test tube. The mixture was then left at 37 °C for 30 minutes. After that, to the mixture, 1 mL of 30% tricholoroacetic acid was added. After that, the mixture was incubated again for 30 minutes. Then, the mixture was centrifuged at 3500 rpm for 15 minutes at 4°C. The absorbance of the filtrate was then measured with a spectrophotometer at a wavelength of 275 nm. Tyrosine levels were analyzed using a linear equation generated from the regression curve that had been created previously. The proteolytic activity of bromelain is then calculated based on the equation below.

$$\text{Enzyme activity (U/mL)} = \frac{\text{Amino acid supernatant} \times \frac{\text{total volume of solution}}{1000}}{1000 \times \text{BM tyrosine}}$$

***Protein Content by Biuret Method using Spectrophotometer*:** Bovine Serum Albumin (BSA) was prepared as the standard. The protein content of the crude enzyme bromelain produced from the extraction process is determined to provide an overview of the enzyme content it contains. BSA standard solutions are made with water solvents with concentrations of 80, 16.0, 240, 320 and 400 ppm. A total of 1 mL of the standard solution was then diluted to a volume of 4 mL and then the mixture was reacted with biuret reagent. The coloured solution formed was then measured with a spectrophotometer with a wavelength of 520 nm. The resulting absorbance is then connected to the concentration in a straight line equation. The absorbance sample was also analyzed by making a 100,000 ppm solution of bromelain from the crude oil. The solution was then centrifuged at 10000

rpm at 4°C for 10 minutes. The supernatant was then transferred into a test tube and reacted with biuret dye. The absorbance of the sample was tested with a spectrophotometer at a wavelength of 520 nm. The bromelain enzyme protein content was calculated based on a comparison of the sample absorption with the BSA absorption standard that had been measured previously.

***Calculation of bromelain levels using HPLC*:** Prior to analysis activity, a standard bromelain solution has been prepared. A standard solution was made by dissolving 100 mg of bromelain and dissolving it into 100 mL of solution in a volumetric flask. Demineralized water solvent was used. The mixture was homogenized using ultrasonic apparatus for 30 minutes at room temperature. The mixture was then transferred into a centrifuge flask and centrifuged to obtain a clear filtrate. The supernatant is then transferred into a vial and ready for injection. The sample solution was also prepared based on the concentration of the standard solution that has been prepared. The analysis used a liquid chromatography method with an HPLC thermos Scientific type Dionex Ultimate 3000 instrument. Analytical separation was carried out using a C18 column with a separation temperature of 30°C. Analytical detection was carried out in the UV area with a wavelength of 280 nm. Analytical separation was carried out using gradient polarity with a mobile phase consisting of 0.1% acetic acid and acetonitrile. The gradient started with 10% acetonitrile and ended until the acetonitrile composition became 30% for 15 minutes. The sample was injected with a volume of 20 micro litres with a mobile phase flow rate of 1 mL/min.

Bromelain content were calculated based on the comparison of the bromelain absorbance obtained in the sample solution with the absorbance of the standard bromelain solution. Standard solutions of bromelain with concentrations of 125, 120, 500 and 1000 ppm have been made using water as a solvent. Each standard solution has been injected into the same chromatography system as the sample solution analysis. The straight-line equation is used to calculate the bromelain content contained in the sample.

***Statistical analysis*:** The multiple range test by Duncan was utilized to assess statistics with a probability threshold of 5%. The R software was used to do the statistical test. The mean observations from three replicates data.

RESULTS

***Yield*:** The yield of crude bromelain produced has a positive linear trend with the length of the pineapple storage process. crude bromelain has increased during the pineapple storage process. The result is shown in Figure 1. The yield of crude bromelain produced ranged from 1.80% to 2.30%. This could be caused by components other than bromelain that increased, because in this study there was no enzyme purification process.

***Protein Content of crude enzyme*:** The findings showed that when pineapples were preserved, the amount of protein in the crude bromelain produced decreased (18.76 g/mL to 6.02 g/mL). The results are shown in Figure 2. This trend is opposite to the yield results. These data suggest that the presence of other components, other than bromelain is responsible for the increase in yield. In addition, when stored, the enzyme may also react with other substances.

***Enzyme Activity*:** The activity of bromelain is affected by the length of storage of pineapple fruit, resulting in a decrease in the value of crude bromelain. Figure 3 illustrates how the variation in estimated enzyme activity values during storage resulted in a value of 27.60 U/mg, which is a considerable decrease from the previous value of 73.73 U/mg. Analysis of crude bromelain showed a decrease in activity. It is important to note that in this case, a low-cost technology was used without going through any purification.

***Bromelain content*:** The amount of bromelain generated was determined by an HPLC analysis. The pineapple's bromelain concentration decreased with storage time, from 2.2435 mg/mL to 0.5175 mg/mL, according to the findings. Figure 4. Shown the findings from bromelain level. It can be seen, for the treatment of days 3, 6 and 9, the difference in bromelain levels is not so significant.

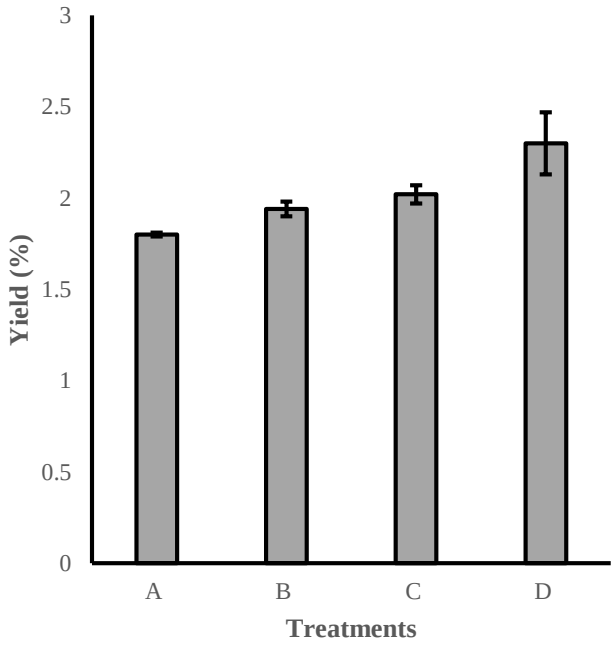

Figure 1. Crude Bromelain Yield

Note: A= day 0 of Pineapple storage, B= days 3 of Pineapple storage, C= days 6 of Pineapple storage, D= days 9 of Pineapple storage. The 5% significance level in the DNMRT test indicates a significant difference.

***Overall results*:** The characteristics of crude bromelain are determined based on the storage time of pineapple fruit (0, 3,

Tabel 1. The effect of pineapple storage time on the characteristics of crude bromelain.

Parameters	Treatments			
	A	B	C	D
Yield (%)	1.80 ± 0.01	1.94 ± 0.04	2.02 ± 0.05	2.30 ± 0.17
Protein content (µg/mL)	18.76 ± 0.41	16.31 ± 0,44	12.93 ± 0.64	6.02 ± 0.77
Enzyme activity (U/mg)	73.73 ± 3.76	33.58 ± 0.72	30.89 ± 0.68	27.60 ± 0.23
Bromelain content (mg/mL)	2.2435 ± 0.053	0.5446 ± 0.016	0.5190 ± 0.023	0.5175 ± 0.034

Note: A= day 0 of Pineapple storage, B= days 3 of Pineapple storage, C= days 6 of Pineapple storage, D= days 9 of Pineapple storage. The 5% significance level in the DNMRT test indicates a significant difference

6, and 9 days) at room temperature. All treatments were evaluated so that differences in the characteristics of the crude bromelain produced could be seen (Table 1.).

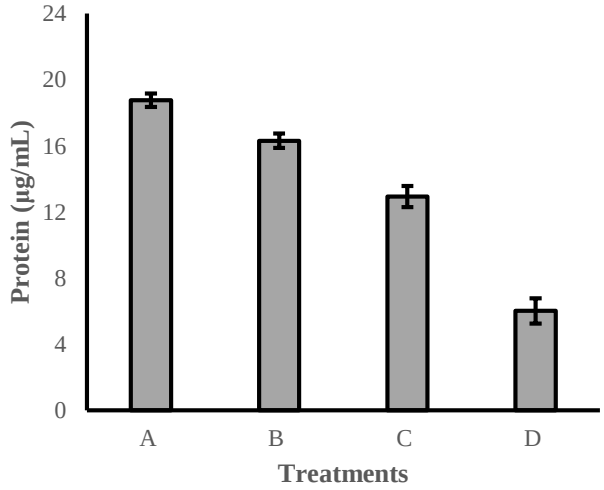

Figure 2. Crude Bromelain Protein Content

Note: A= day 0 of Pineapple storage, B= days 3 of Pineapple storage, C= days 6 of Pineapple storage, D= days 9 of Pineapple storage. The 5% significance level in the DNMRT test indicates a significant difference.

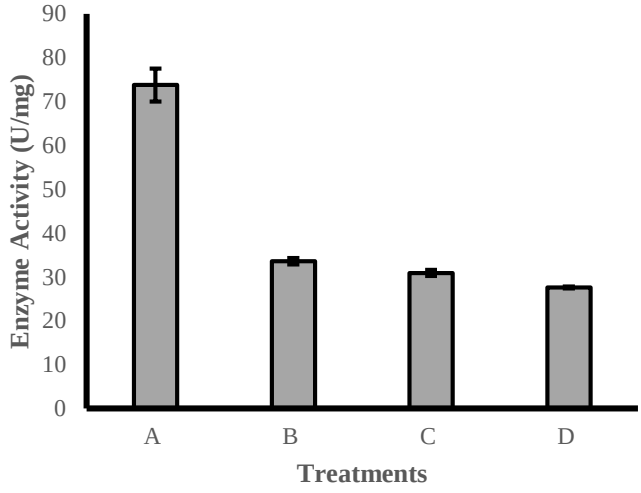

Figure 3. Crude Bromelain Activity

Note: A= day 0 of Pineapple storage, B= days 3 of Pineapple storage, C= days 6 of Pineapple storage, D= days 9 of Pineapple storage. The 5% significance level in the DNMRT test indicates a significant difference.

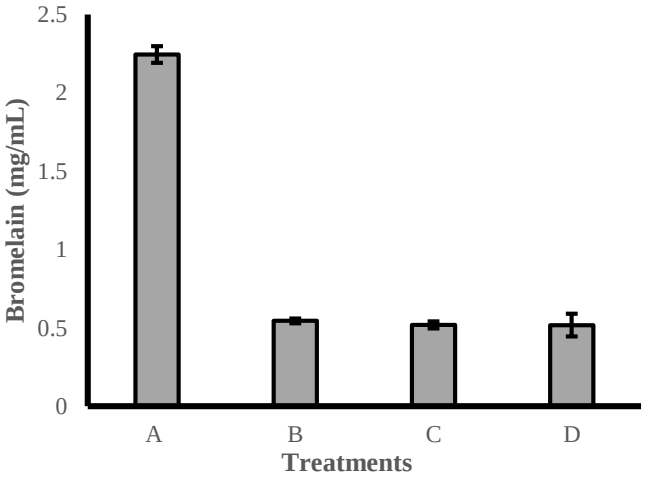

Figure 4. Bromelain Content

Note: A= day 0 of Pineapple storage, B= days 3 of Pineapple storage, C= days 6 of Pineapple storage, D= days 9 of Pineapple storage. The 5% significance level in the DNMRT test indicates a significant difference.

DISCUSSION

The pineapple storage process can affect the characteristics of the crude bromelain produced, both in terms of yield, protein content, enzyme activity and bromelain content. The yield of crude bromelain has increased. This finding is consistent with Poba *et al.* (2019), who discovered that as pineapple matures, the amount of crude bromelain increased due to other components increasing during the metabolic process (Dhandayuthapani *et al.*, 2012). According to Ware (Ware and Olsen, 2017), pineapple fruit that is still fresh or ready to harvest has more bromelain, around 2.31% compared to fruit that has been stored longer.

Furthermore, the observed discrepancy might originate from the significant quantity of additional constituents present in crude bromelain, which is generated via intensifying the ripening process and decomposition of pineapple fruit while it is in storage, hence inflating the output. Pineapple sugar is

another ingredient that has the potential to raise one of them. Alcohol cannot entirely dissolve it (Kessler *et al.*, 2013), although 90% alcohol was used in this study's extraction procedure. Research by (Techavuthiporn *et al.*, 2017), which found that pineapple sugar content rose from 10% (day 0) to 40% (storage day 8) at 25 °C, lends credence to this finding.

In contrast to the yield, the protein content, enzyme activity and bromelain content of crude bromelain decreased. The research by Tapre and Jain (Tapre and Jain, 2012), which found that long storage of fruit can also reduce protein levels strengthens this conclusion. Not only that, it shows that crude bromelain contains significant impurities. Ethylene formation during respiration, transpiration, and other physiological processes can also chemically react with cell enzymes to cause this deterioration (Hikisz and Bernasinska-Slomczewska, 2021). Complex substrates including proteins, lipids, and carbohydrates are broken down during respiration into components of CO_2, H_2O, and simple energy (Rahmadhanni *et al.*, 2020).

The decrease in enzyme activity in this study could also be caused by changes in pH and moisture content in pineapple juice. pH, temperature, substrate, and inhibitor concentration are the main variables that affect enzyme activity (Soares *et al.*, 2011). The pH of pineapple juice will rise from 3.9 to 4.5 the during pineapple is stored. According to Chia *et al.* (2012) and Ngozi *et al. (*2010) an increase in pH can cause an enzyme's activity to decrease. Furthermore, a drop in the crude bromelain's protein content contributed to the decline in enzyme activity. The activity of bromelain generated can also be decreased by pineapple rotting. This result is consistent with that of Poba *et al.* (2019), which found that bromelain activity decreased with higher levels of pineapple fruit maturity due to metabolic processes in the fruit and an increase in other impurities such as sugar.

Bromelain content in crude bromelain decreased. This data was consistent with the protein content (Figure 2), which shows that as storage times increase, the amount of crude enzyme produced decreases in terms of protein content. Soares *et al.* (2011) stated that impurities and the physical similarity of proteins in the same solution are the reasons for the low concentration of enzymes in crude bromelain. Additionally, this data demonstrates that during pineapple storage, the amount of components other than bromelain has grown, such as sugar content.

***Conclusions*:** The characteristics of crude bromelain produced depend on how long the pineapple is stored. From day 0 to day 9, crude bromelain production in pineapple storage increased from 1.80% to 2.30%. Protein content decreased from 18.76 g/mL to 6.02 g/mL, crude bromelain activity level decreased from 73.73 U/mg to 27.60 U/mg, and bromelain content decreased from 2.2435 mg/mL to 0.5175 mg/mL. crude bromelain produced in stored pineapple can certainly still be used in different grades. in the future, further purification of bromelain can be done. As information, pineapple storage is likely to occur in retail sales and community distribution. Therefore, the data resulting from this research will be used as a practical reference in the future, where from this research it can be seen that pineapples that have not been sold for 9 days can still be used. Pineapples that are almost rotten can still be processed to produce bromelain. Bromalain is an enzyme that can be used in wider and longer applications.

***CRediT author statement*:** Ririn Fatma Nanda: wrote the manuscript; Anwar Kasim: reviewed the manuscript; Rini: data analysis; Daimon Syukri: conception the research; Ira Desri Rahmi: statistical analysis; Reni Koja: laboratory analysis; Kohei Nakano: Research Supervisor; Manasikan Thammawong: Research co-Supervisor

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***SDGs addressed*:** Responsible Consumption and Production, Industry, Innovation and Infrastructure, Climate Action, Zero Hunger.

***Policy referred*:** Agricultural Waste Utilization Policy; Circular Economy and Bioeconomy Policy; Postharvest Loss Reduction Strategy; Smallholder Farmer and Rural Enterprise Development.

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